REVIEW 10.1111/j.1469-0691.2004.00895.x

Lyme borreliosis: from infection to autoimmunity

S. K. Singh and H. J. Girschick

Paediatric Rheumatology, Children's Hospital, University of Würzburg, Würzburg, Germany

ABSTRACT

Lyme borreliosis in humans is an inflammatory disease affecting multiple organ systems, including the nervous system, cardiovascular system, joints and muscles. The causative agent, the spirochaete *Borrelia burgdorferi*, is transmitted to the host by a tick bite. The pathogenesis of the disease in its early stages is associated largely with the presence of viable bacteria at the site of inflammation, whereas in the later stages of disease, autoimmune features seem to contribute significantly. In addition, it has been suggested that chronic persistence of *B. burgdorferi* in affected tissues is of pathogenic relevance. Long-term exposure of the host immune system to spirochaetes and/or borrelial compounds may induce chronic autoimmune disease. The study of bacterium–host interactions has revealed a variety of proinflammatory and also immunomodulatory–immunosuppressive features caused by the pathogen. Therapeutic strategies using antibiotics are generally successful, but chronic disease may require immunosuppressive treatment. Effective and safe vaccines using recombinant outer surface protein A have been developed, but have not been propagated because of fears that autoimmunity might be induced. Nevertheless, new insights into the modes of transmission of *B. burgdorferi* to the warm-blooded host have been generated by studying the action of these vaccines.

Keywords *Borrelia burgdorferi*, review, spirochaetal persistence, tick-borne disease, virulence

Accepted: 5 November 2003

Clin Microbiol Infect 2004; 10: 598–614

INTRODUCTION

Lyme borreliosis is an inflammatory disease caused by the spirochaete *Borrelia burgdorferi*. This Gram-negative microorganism is transmitted to a variety of hosts by ticks, mainly of the genus *Ixodes*. Lyme borreliosis in humans manifests as a multisystem disorder of the skin and other organs, such as joints, cardiac system, nervous system and eyes.

Since surveillance for Lyme disease was begun by the Centers for Disease Control in 1982, the number of cases reported each year in the USA has increased dramatically to about 15 000, making Lyme disease the most common vector-borne disease in the USA [1]. In Europe, Lyme borreliosis has been documented widely in forested areas. The highest frequencies of the disease have been reported from Scandinavia, Germany, Austria, Slovenia and Sweden [2]. The infection has also been reported in Russia, China and Japan.

Ticks of the genus *Ixodes* undergo larval, nymphal and adult stages during their life cycle. The risk of infection in a given area depends largely on the environmental density of ticks, their feeding habits and their animal hosts. Small mammals, particularly rodents, are important hosts of ticks and are critical for maintenance of *B. burgdorferi* in nature. In addition, deer serve as hosts, especially during the adult tick stages [3,4]. The predominant tick species that transmit *B. burgdorferi* to humans are the Eurasian species *Ixodes ricinus* and *I. persulcatus*, and the North American species *I. dammini*, *I. pacificus* and *I. scapularis* [5]. The larval stage of the insect that emerges from the egg passes through a nymphal stage before developing into an adult.

The tick feeds only once at each stage in its life cycle. Activity of the ticks is seasonal. The tick bite is usually painless, and therefore may go unnoticed. Indeed, more than half of affected individuals do not remember a tick bite. During feeding, the tick may transmit *B. burgdorferi* through its

Corresponding author and reprint requests: H. Girschick, Children's Hospital, University of Würzburg, Josef-Schneider-Str. 2, 97080 Würzburg, Germany
E-mail: Hermann.Girschick@mail.uni-wuerzburg.de

saliva. *B. burgdorferi* is distributed in the mid-gut of the infected ticks. It is thought that, once the tick has its blood meal, *B. burgdorferi* penetrates the gut mucosa, disseminates into other tissues, including the salivary gland, and is inoculated into the host within 12–72 h [6].

In Europe, the proportion of ticks reported to harbour *B. burgdorferi* ranges from 0% to 85%; in the USA, it varies from 1% to 100%. This percentage depends on the developmental stage and the prevalence of infection of the ticks. Infection is highest in the adult and nymph forms of the tick, and lowest among the larval forms [7]. Ovarian transmission of *B. burgdorferi* from the mother tick to the offspring is possible [8]. *Borrelia* can invade the developing oocyte yolk complex in the ovary from the haemolymph before the impervious shell forms around the egg [9,10]. During embryonic development, spirochaetes can migrate from the yolk region to neuronal ganglia. Ovarian transmission in ticks can be very efficient, with passages over five to nine generations of ticks having been documented [10].

BIOLOGY OF *BORRELIA BURGDORFERI*

The aetiological agent *B. burgdorferi sensu lato* has been subdivided into three species causing human Lyme disease: *B. burgdorferi sensu strictu*, *B. afzelli* and *B. garinii*. Strains of all three species have been isolated from patients in Europe, whereas only the first species has been reported in the USA.

Borrelia spp. vary in length, diameter, tightness of the coils, and number of periplasmic flagella. The length can range from 10 to 30 μm and the width of the helices from 0.2 to 0.25 μm [11]. The generation time under optimal conditions (30–40°C, microaerophilic) is 7–20 h. Ultrastructurally, the causative agent resembles other spirochaetes of the genus *Borrelia*, with a non-patterned surface layer, a three-layered outer-membrane surrounding a periplasmic space containing the variable number of flagella, and the protoplasmic cylinder [12] (Fig. 1).

B. burgdorferi has a linear chromosome in addition to linear and circular plasmids. The genes that encode major outer-surface proteins (Osps) are located on plasmids [13]. *B. burgdorferi* strains usually have three major Osps (OspA, OspB, OspC) [13], along with OspD [14], OspE, OspF [15] and OspG.

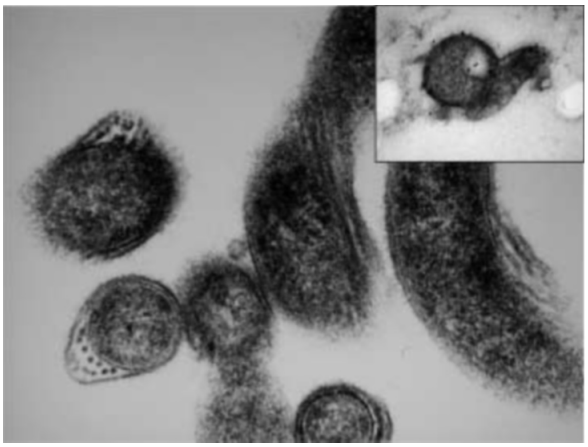

Fig. 1. Transmission electronmicrograph of *Borrelia burgdorferi* strain *sensu strictu* showing the corkscrew shape and motility, and the outer-membrane surrounding a periplasmic space that contains the variable number of flagella and the protoplasmic cylinder. Insert: Immuno-electronmicrograph using a polyclonal antiserum against *Borrelia burgdorferi*, demonstrating that a bleb formed during in-vitro culture is an integral part of the bacterial cell.

The loose association of the outer-envelope with the underlying protoplasmic cylinder leads to the separation of the cell components when borrelia are kept in hypotonic solution [16]. This so-called bleb formation has been observed on the outer-envelope of *Borrelia in vitro* when a specific antibody or complement is added [17] (Fig. 1). Studies have indicated that exposure to physiological stress, e.g., changes in pH, depletion of metabolites and ageing [17], or exposure to antibiotics [18,19], can lead to bleb formation. Little is known about the physiological role of these forms, although blebs and gemmae have been shown to contain DNA [20] and may be involved in the exchange of genetic information.

Alban *et al.* [21] reported that serum starvation resulted in the transformation of motile helical forms into non-motile, spherical cysts containing tightly coiled spirochaetes. Addition of tetracycline inhibited the formation of cysts, demonstrating that cyst formation requires protein synthesis. Unlike cyst forms, blebs and gemmae have not been shown to be viable, and seem to be incapable of transforming back into motile vegetative bacteria [21]. Cysts have been observed in human cerebrospinal fluid (CSF) [22] and in the tissues of patients infected with Lyme disease [23,24]. Thus, if viable cysts can form in the human body, they may represent a strategy that facilitates the survival of *B. burgdorferi* during

nutritionally adverse conditions in host tissues. By forming cysts, it is also conceivable that *B. burgdorferi* might evade detection by the host immune system [21]. Hulinska *et al.* [24] demonstrated that the surface of cysts found in erythema migrans of infected human tissue was non-reactive to antibodies against OspA, whereas the content of the cysts did react with OspA [24].

Periplasmic flagella can be found beneath the outer-membrane, and these mediate spirochaete motility. Borrelial flagella resemble one another and basically have the same structural organisation as the flagella of other eubacteria [25]. Like other spirochaete flagella, borrelial flagella also insert sub-terminally and with bipolarity. Different *Borrelia* spp. have different numbers of flagella [26]. The flagella have four components—the filament, the hook, the neck and the basal disk [27]—and resemble flagella of Gram-negative bacteria [28]. While treponemal flagella are sheathed, borrelial flagella are characteristically unsheathed [29] and are composed predominantly of flagellin protein with a molecular mass of 41 kDa [30].

The borrelial cell membrane contains muramic acid [31] and ornithine as a diamino acid in a peptidoglycan structure [32]. A unique feature of *Borrelia* spp. is that the genes for the immunodominant outer-surface proteins OspA, OspB and OspC are located on plasmids. Although the outer-surface proteins are immunologically and genetically variable [33], OspA [34] and OspC [35] have proven to be very useful proteins for the development of a *Borrelia* vaccine. Other important antigens of *B. burgdorferi* are the flagellin protein and a 60-kDa antigen. The latter has been termed a common antigen and belongs to the heat-shock protein family. The flagellin protein and the 60-kDa antigen are not species-specific.

The complete genome of *B. burgdorferi sensu strictu* (strain B31) has now been sequenced [3]. The genome is quite small (*c.* 1.5 Mb) and consists of a highly unusual linear chromosome of 950 kb, together with nine linear and 12 circular plasmids. The outer-surface proteins of *B. burgdorferi* presumably help the spirochaete to adapt to and survive in markedly different environments between cold-blooded ticks and mammals [4].

B. burgdorferi undergoes dramatic metamorphosis, including changes in expression of many different lipoproteins, when it transfers from the tick to the mammalian environment. Inside the mid-gut of unfed ticks, *B. burgdorferi* expresses the OspA lipoprotein in large amounts [36]. Immunofluorescence studies have shown that after the tick attaches to the mammalian host and begins feeding, *B. burgdorferi* starts to down-regulate OspA expression, and rapid synthesis of OspC begins [37]. At the same time, *B. burgdorferi* migrates from the tick mid-gut to the salivary glands in preparation for mammalian host infection. Once *B. burgdorferi* is inside the mammalian host, the transition from OspA to OspC expression is complete. Now *B. burgdorferi* no longer expresses OspA, while OspC is detectable readily on spirochaetes that have adapted to the mammalian host [38].

Temperature adaptation is one important factor that *B. burgdorferi* uses to undergo this transition. Changes in lipoprotein expression can also be demonstrated by comparing *B. burgdorferi* grown in culture at 23–24°C with that grown at 35°C [37]. The effects of temperature on the differential expression of OspA and OspC are enhanced by co-cultivation of *B. burgdorferi* with tick cells [39]. In addition to OspC, other lipoproteins are also up-regulated during the transition to the warm-blooded mammalian host. Thus, when culture conditions are changed from low to high temperatures, up-regulated expression of the two outer-surface proteins OspE and OspF can be observed [40]. It has become evident that spirochaetal proteins, up-regulated during infection of mammals, are members of a large family of OspE- and OspF-related proteins, currently designated Erps [41]. Further studies have shown that expression of Erp proteins can be induced in the cultivated *B. burgdorferi* strain B31 by a shift in temperature from 23°C to 35°C [40].

Incubation of spirochaetes at different temperatures is one method of mimicking environmental signals that can regulate lipoprotein expression. In addition, another surface-exposed lipoprotein has been reported to undergo extensive antigenic variation during early disseminated infection [42]. The genome does not contain any homologue for the system that enables the organism to secrete toxins and other virulence factors. So far, the only known virulence factors of *B. burgdorferi* are surface proteins that allow the spirochaete to attach to mammalian cells. The organism produces few proteins with biosynthetic activity, and apparently depends on the host for many of its nutritional requirements.

CLINICAL FEATURES OF LYME DISEASE

Lyme borreliosis in adults can be divided into three clinical stages [43–45] that may overlap with each other. The first two stages, which appear within a few weeks or months after infection with *B. burgdorferi*, represent the early phase of the disease. The third, or late, phase appears after several months or years [46]. The early features of the disease are usually self-limiting, but late features may become chronic and progressive. Previous exposure to *B. burgdorferi* does not prevent infection; indeed, the occurrence of erythema migrans in a patient with acrodermatitis chronica atrophicans has been reported [44].

Stage I: Early infection

In the early stage of infection, erythema migrans develops regularly, but can be absent in up to 50% of patients (for details of the clinical morphology, see [45]). The skin lesion is frequently accompanied by influenza-like symptoms, such as malaise, fatigue, headache, fever and regional lymphadenopathy. In Europe, erythema migrans often remains an indolent localised infection, whereas in the USA this lesion has been associated with more intense inflammation and signs that often suggest early dissemination of the spirochaete [47]. In one study, spirochaetes could be cultured from plasma samples in 50% of patients affected with erythema migrans [48]. The lymphocytoma, another acute skin lesion, appears typically on the nipple or areola of the breast [49], or on the ear lobes [45]. These lesions are usually caused by *B. afzelli* or *B. garinii* [50], and are therefore reported mainly in Europe, but not in the USA. A few weeks to months after infection, several organs may become affected, probably because of haematogenous spread of the pathogen.

Stage II: Early dissemination

After a tick bite, *B. burgdorferi* might spread from the site of the bite into the bloodstream, causing clinical signs of early dissemination. Lyme disease can affect the nervous system. Neural manifestations during this stage are meningoradiculoneuritis (Bannwarth's syndrome), meningitis, plexus neuritis, cranial neuritis (predominantly involving facial nerves) and mononeuritis multiplex. Bannwarth's syndrome, which in Europe is the most common neurological manifestation in adults, is characterised by CSF lymphocyte pleocytosis and intense radicular pain. The spread of *B. burgdorferi* within the nervous system has been demonstrated in non-human primates [51], the only known model of neuroborreliosis. In immunocompromised monkeys, which have a larger spirochaetal burden than immunocompetent animals, *B. burgdorferi* has been shown to infiltrate leptomeninges, sensory and motor nerve roots, and the dorsal root ganglion, but not the brain parenchyma [52]. In the peripheral nervous system, spirochaetes have been detected in the perineurium, the connective tissue sheath surrounding each bundle of peripheral nerve fibres.

Few patients suffering from Lyme disease develop heart problems. Most commonly, atrioventricular blockage has been described, and occasionally acute myopericarditis or mild left ventricular dysfunction, but rarely cardiomegaly [43]. These heart abnormalities can appear several weeks after infection. In Europe, *B. burgdorferi* has been isolated from endomyocardial biopsy samples from several patients with chronic dilated cardiomyopathy [53].

Arthralgia and myalgia indicate early musculoskeletal involvement. Frank arthritis and myositis can also be observed occasionally in the first few months of the disease. Regional lymphadenopathy and generalised lymphadenopathy may develop.

Stage III: Chronic disease

Chronic organ involvement may develop years after the tick bite. Skin and soft tissue manifestations are common in Lyme borreliosis [45]. Acrodermatitis chronica atrophicans starts as an inflammatory dermatitis. Later, this lesion evolves into an atrophic skin lesion.

Among untreated patients in the USA, *c.* 60% begin to have intermittent attacks of joint swelling and pain. Large joints, especially the knees, are primarily affected [54]. Synovial tissue from affected patients shows synovial hypertrophy, vascular proliferation and marked infiltration of mononuclear cells. Furthermore, patients with Lyme borreliosis usually have higher *Borrelia*-specific antibody titres in serum than do patients with any other manifestation of the disease. After several short attacks of arthritis, some patients may develop persistent joint inflammation.

Direct involvement of the eye (keratitis, optic neuritis) has also been attributed to *B. burgdorferi* infection [43,55]. However, since *B. burgdorferi* has been isolated rarely from patients with these opthalmological disorders, the pathogenesis in these cases is uncertain.

In both the USA and Europe, a chronic axonal polyneuropathy may develop, manifested primarily as spinal radicular pain [56,57]. Electromyograms typically show diffuse involvement of proximal and distal nerve segments. In Europe, *B. garinii* may cause chronic encephalomyelitis, characterised by cranial neuropathy with marked intrathecal production of antibodies against the spirochaetes [56]. In the USA, a mild late neurological syndrome has been reported, termed Lyme encephalopathy. It is characterised by memory deficit, minor depression, irritability and somnolence [58–61].

The differences between genospecies found in Europe and North America may account for differences in the frequencies of certain manifestations of Lyme disease in these areas. For example, neurobiological manifestations of Lyme disease are more common in Europe, whereas rheumatological manifestations are more common in North America.

Perinatal Lyme disease

Case reports have suggested that adverse outcomes of pregnancies may be complicated by maternal Lyme borreliosis [62]. The risk of transplacental transmission of *B. burgdorferi* is probably minimal when appropriate antibiotics are given to a pregnant woman with Lyme borreliosis. There have been several published case series investigating the relationship between gestational Lyme disease and fetal outcome. The questions addressed in these series were as follows. Does *B. burgdorferi* cross the placenta and invade the foetus? If there is transplacental transmission, does this have any significance for the development of the foetus? Several studies have shown a relationship between seropositivity in pregnancy and pregnancy outcome. A large serological study of 2014 women, of whom 12 were seropositive, revealed no increased risk of congenital malformations, low birth weight, abnormal length of gestation or risk of fetal death among children born to seropositive mothers [63]. A second study of 1416 pregnant women, of whom 12 were seropositive at delivery, also revealed no adverse outcomes attributable to seropositivity [64]. A study comparing 5000 infants, divided equally between a Lyme-endemic area and a control area, showed no significant differences in the incidence of congenital malformations, except for a statistically significant increase in the rate of cardiac malformations in the Lyme-endemic area [65]. However, it is not known whether this finding represents an artefact or a valid difference between the two populations. A further epidemiological study conducted in a Lyme-endemic area has questioned the connection between maternal Lyme disease and congenital heart disease [66]. In this study of 796 patients and 704 control subjects, there was no significant association between congenital defects and maternal Lyme disease. In another clinical study, a higher incidence of neurological disorders was not found in children of women with gestational Lyme disease in an epidemic area.

Studies in both human and animal models have established that *B. burgdorferi* can cross the placenta, presumably during the period of initial spirochaetaemia. Despite documentation of transplacental transmission of *B. burgdorferi*, there has been no clinical evidence for a fetal inflammatory or immune response, or an adverse neonatal outcome resulting from gestational Lyme disease [67]. The above studies indicated that an adverse fetal outcome resulting from maternal infection with *B. burgdorferi* at any point during pregnancy in humans is, at most, extremely rare.

Lyme disease in children

In principle, manifestations among children are the same as in adults. However, Lyme disease in children follows a somewhat different course and has different symptoms than in adults (Table 1). In most cases, the clinical and epidemiological features are comparable among children suffering from Lyme disease in Europe and the USA [68,69]. Children account for a relatively high number of Lyme borreliosis patients, presumably because of greater exposure to ticks and decreased attention to prevention of Lyme disease. Frequently, tick bites in children occur on the upper parts of the body, and especially on the head. Thus, modes of pathogen dissemination inside the host, especially into the central nervous system, might occur faster than in adults. This is reflected by the fact that

Table 1. Comparison of Lyme disease manifestations in children and adults

Course of disease	Children	Adults
Early manifestations (days to a few weeks)		
General symptoms	Influenza-like disease	Influenza-like disease, lymphadenopathy
Skin	Erythema migrans, lymphocytoma	Erythema migrans, lymphocytoma
Neurological	Lymphocytic meningitis	
	Cranial neuritis, mainly the facial nerve	
Heart	Myopericarditis	
Eye	Conjunctivitis	
Joint; muscle	Arthralgias	
Early dissemination (after a few weeks)		
General symptoms		Lymphadenopathy
Neurological		Meningitis
		Meningoradiculoneuritis (Bannwarth's syndrome)
		Plexus neuritis, cranial neuritis
		Mononeuritis multiplex
Heart		Atrioventricular blockage
		Myopericarditis, cardiomyopathy
Joint; muscle		Arthralgia, myalgia, oligoarthritis
Late stage of infection, chronic disease (months to years after infection)		
Skin	Acrodermatitis chronica atrophicans (rare)	Acrodermatitis chronica atrophicans
Neurological	Meningoradiculoneuritis (rare)	Axonal, sensory polyneuropathy
	Encephalomyelitis (rare)	Cranial neuropathy
		Chronic encephalomyelitis, encephalopathy Cardiomyopathy (rare)
	Heart	Cardiomyopathy
Eye	Uveitis, keratitis	Retinitis, uvetis, keratitis, endophthalmitis
Joint; muscle	Episodic or chronic oligoarthritis	Treatment-resistant arthritis

meningitis in children occurs frequently early in the course of the disease, and often in parallel with erythema migrans. Lymphocytic meningitis, with or without cranial neuropathy, is the major early neurological manifestation of Lyme disease in children, and generally presents with episodic headache, mild neck stiffness, numbness and poor motor co-ordination.

Eppes *et al.* [70] compared Lyme meningitis with viral meningitis in children. Like Lyme meningitis, viral meningitis occurs during summer, and is difficult to diagnose in Lyme-endemic regions, but it was suggested that some clinical and laboratory findings in Lyme meningitis are sufficiently distinctive: (1) cranial neuropathy, especially peripheral seventh nerve palsy, is strong evidence of Lyme meningitis; (2) papilloedema is more likely to be seen in Lyme meningitis than in viral meningitis; (3) a longer duration of symptoms before hospital admission supports a diagnosis of Lyme meningitis; (4) fever at the time of diagnosis is more likely to be related to viral meningitis; and (5) CSF pleocytosis is less pronounced in Lyme meningitis than in viral meningitis, and especially the initial neutrophilic component [70]. Chronic late stage neurological disease, e.g., meningoradiculoneuritis or encephalomyelitis, is rare and is not as frequent as joint disease [62].

Lyme borreliosis may be the cause of a variety of inflammatory changes of the eye occurring in childhood, either early (conjunctivitis) or late (uveitis, keratitis) [55]. In a German study of 84 children with arthritis caused by late Lyme borreliosis, three had borrelial eye disease, including keratitis, anterior uveitis and uveitis intermedia [71]. Clinicians should be aware that optic nerve involvement may be a manifestation of Lyme disease, because of either inflammation or increased intracranial pressure, or both [55]. Among patients with symptoms suggestive of Lyme disease, decreased vision suggests the possibility of optic neuritis, whereas the presence of headache, visual symptoms, pulsatile tinnitus, sixth nerve palsy or papilloedema can be important signs of increased intracranial pressure. A few children in the USA have been reported with optic neuritis associated with Lyme disease, which occurred 1–9 months after initial infection [55,72–75].

Younger children (aged < 10 years) are more likely to have fever at onset of arthritis, followed by an acute or episodic course, and to have lower antibody titres to *B. burgdorferi* compared to adults [76,77]. It has been documented that the clinical characteristics of children with Lyme arthritis vary with age. Among adults with Lyme arthritis,

c. 10% are reported to develop chronic arthritis. In children with Lyme arthritis, a chronic course of arthritis seems less frequent [78,79]; however, in a European study, 24% of children affected by Lyme arthritis retained manifestations of disease, including arthritis and arthralgias, 12 months after antibiotic treatment [76]. In contrast to adult patients, temporomandibular or sternoclavicular joint involvement is rare in children.

PATHOGENESIS

Pathogenic factors encoded by spirochaetes

Most of the sub-surface lipoproteins of *B. burgdorferi* play an important role in cellular physiology and participate directly in pathogenesis [3,80]. Mutants deficient in OspA are more sensitive to complement lysis and digestion by proteases in the mid-gut of ticks [81,82]. These facts suggest that OspA expression by *B. burgdorferi* during tick infection might protect the organism both from tick mid-gut proteases and from mammalian complement when the tick takes blood from the host. The ability of spirochaetes to attach to eukaryotic cell surfaces and extracellular matrix proteins is also essential in pathogenesis.

Several pathogenic mechanisms may aid in the dissemination of *B. burgdorferi*. For example, the sequences of OspC differ considerably among *Borrelia* strains, and only a few particular sequences have been associated with dissemination of disease [83]. Spread through skin and other tissue matrices may be facilitated by the binding of human plasminogen and its activators to the surface of the spirochaete [84]. During the dissemination and homing of *B. burgdorferi* to specific sites, the pathogen attaches to certain host integrins [85], matrix glycosaminoglycans [86] and extracellular matrix proteins [87].

Decorin is a collagen-associated glycosaminoglycan found in various tissues, including skin and joints, i.e., sites typically associated with Lyme disease. *B. burgdorferi* has been shown to attach selectively to decorin, which led to the subsequent isolation of decorin-binding protein (Dbp) genes by screening an expression library with digoxigenin-labelled decorin [86,88]. The two gene operons encoding the lipoprotein DbpA and the related DbpB were identified independently by screening a *B. burgdorferi* expression library [89,90] and by sequencing DbpA found in *B. burgdorferi* outer-membranes [91]. *Borrelia* decorin-binding proteins A and B bind decorin on collagen fibrils, which may explain why the organism is commonly aligned with collagen fibrils in the extracellular matrix of the heart, nervous system and joints. In a recent report, decorin-deficient mice had more limited spirochaetal colonisation of joints, and milder arthritis, than normal mice of the same strain that expressed decorin [92]. DbpA demonstrates considerable heterogeneity among *B. burgdorferi* strains. Chemical modification of lysine residues was found to abrogate DbpA binding to decorin.

Involvement of the infected host

Several studies have suggested that *B. burgdorferi* is present at the site of inflammation in many clinical manifestations of Lyme disease. *B. burgdorferi* does not produce toxins, but is a potent immunomodulator. The effects of *B. burgdorferi* on local cells may lead to suppression of the local immune response. A decrease in the expression of major histocompatibility complex (MHC) markers on Langerhans' cells in the skin of patients with acrodermatitis chronica atrophicans has been demonstrated after contact with *Borrelia* [93].

B. burgdorferi infection has been shown to increase the expression of neural cell adhesion molecule on endothelial cells in comparison with controls [94]. After *B. burgdorferi* has entered the skin, it can move through the extracellular matrix. *B. burgdorferi* can bind to the components of the extracellular matrix, including epithelial cell-derived proteoglycans, by interacting with decorin [88], glycosaminoglycans [95] and fibronectin [96,97]. Many studies have shown that *B. burgdorferi* can attach to human umbilical vein endothelial cells (HUVECs) *in vitro*, and that it can traverse HUVEC monolayers grown on tissue substrate. This penetration may be either between or through the HUVECs [98]. HUVECs exposed to *B. burgdorferi* in in-vitro culture show increased expression of E-selectin, vascular cell adhesion molecule and intercellular cell adhesion molecule.

Other resident joint and vascular endothelial cells seem to react differently. Synovial fibroblasts have been shown to down-regulate intercellular cell adhesion molecule-1 after contact with *B. burgdorferi*. Vascular cell adhesion molecule expression was not changed in these cells after

infection with *B. burgdorferi.* In addition, the expression of nitric oxide synthase was not altered by the infection [99]. It is of particular interest that synoviocytes strongly up-regulate these molecules during an inflammatory response. Thus, these local immunomodulatory features might be sequelae of *B. burgdorferi* residing, or even persisting, in the cytosol of resident joint cells, as has been demonstrated *in vitro* (Fig. 2) [100]. However, intracellular persistence of a spirochaete has not so far been demonstrated consistently *in vivo*.

Despite robust humoral and cellular immunity against *B. burgdorferi* in most patients, the disease can become chronic, even after several courses of antibiotic treatment. The pathogenesis of chronic Lyme disease remains a topic of discussion, currently focusing on the concepts of persistent infection and/or autoimmunity. Recently, chronic joint inflammation has been attributed to autoimmunity [101,102].

There is strong evidence that T-lymphocytes play a major role in the pathogenesis of Lyme arthritis. It has been shown that the proliferative response of *B. burgdorferi*-specific T-lymphocytes isolated from the synovial fluid or peripheral blood of adults [103–107] and children with Lyme arthritis [108,109] is elevated. In addition, T-lymphocytes from individuals with prolonged Lyme arthritis responded more vigorously to stimulation with *B. burgdorferi* antigens than the T-lymphocytes from adult individuals affected by less progressive forms of Lyme arthritis [110,111]. Likewise, cloned T-lymphocytes from a patient with chronic Lyme arthritis exhibited enhanced proliferative responses to various *B. burgdorferi* antigens [112].

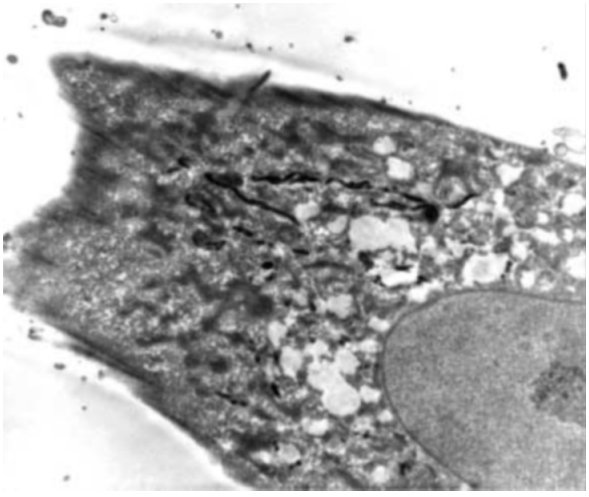

Fig. 2. Transmission electronmicrograph of a human synovial cell infected with *Borrelia burgdorferi in vitro*, demonstrating an intracellular cytosolic location of the spirochaetes, identified by multiple corkscrew-shaped structures in the cytosol.

Many investigators have demonstrated that individuals with progressive forms of Lyme arthritis frequently have T-lymphocytes that liberate lymphokines characteristic of the Th1 phenotype [112,113]. The existence of highly polarised Th1 lymphokine patterns has also been described in mice infected with *B. burgdorferi*. Other studies [114–116] have shown a relationship between T-cell phenotypes and the induction or control of Lyme arthritis. T-cell involvement in the pathogenesis of Lyme arthritis has been described in a hamster model of Lyme borreliosis. Lymph node T-cells from hamsters vaccinated with 10^8 formaldehyde-inactivated spirochaetes in adjuvant are able to confer susceptibility to severe Lyme arthritis when transferred into naive hamsters challenged with 10^6 viable *B. burgdorferi* cells [114]. When primed T-cells were infused into naive recipients and challenged with dead *B. burgdorferi*, no arthritis was seen [114].

These studies suggest that gene products expressed actively by the spirochaete inside a mammalian host might be involved in the propagation of Lyme arthritis mediated by T-cells [117]. Hence, T-cells seem to be responsible for the development of destructive Lyme arthritis [118]. However, protection from disease by $CD8^+$ T-cells has been reported [116].

In the initial phases of disease, humoral immune responses in general are limited, or at least specific antibodies are hard to detect [119]. B-cell reaction to the pathogen might be abrogated by early antibiotic treatment [120]. Therefore, serological testing after early antibiotic treatment of erythema migrans is mostly unremarkable and is not recommended [121]. In the chronic phases of disease, a robust B-cell response against a variety of *Borrelia* epitopes is generally detectable [122] and can be used for serodiagnosis. However, this humoral response seems not to be protective, since it cannot prevent reinfection of the host with *B. burgdorferi*. Children and adults do not differ in their antibody response against *B. burgdorferi* [123]. The role of the B-cell response and antibody production in the pathogenesis of chronic Lyme disease is still to be elucidated.

It is well-established that T-lymphocytes of the Th1 phenotype are involved in the activation of

macrophages [124]. Macrophage activation may be central to the induction of Lyme arthritis [125,126]. Elevated levels of various macrophage-derived molecules (interleukin-1, tumour necrosis factor, prostaglandin E2 and collagenase) have been detected in synovial fluid extracts and serum from individuals with Lyme arthritis [127–130]. Interleukin-1 and tumour necrosis factor are known to activate osteoclastic cartilage and bone reabsorption, and may be responsible for the clinical and pathological manifestations of Lyme arthritis [131]. Macrophages can also be stimulated with *B. burgdorferi* to produce nitric oxide [132]. Production of nitric oxide has been associated with the induction of other arthritic diseases [133,134].

Autoimmune features of Lyme disease

One potential explanation for antibiotic-resistant Lyme disease is the generation of autoimmunity directly or indirectly mediated by the pathogen and based on molecular mimicry. Gajdusek [135] has suggested that axonopathy in a variety of neurological diseases might result from anti-axonal antibody production, and anti-axonal IgM antibodies have been demonstrated in the serum of patients with neurological Lyme disease [136]. Genetic linkage studies in adults with Lyme arthritis have demonstrated a link with MHC class II molecules DR2 and DR4 [137]. In addition, these patients develop anti-OspA antibodies correlating with the duration of their arthritis [138], suggesting that OspA may be involved in the autoimmune process. Self-reactive T-cells may maintain local inflammation [139].

MHC class II molecules play a critical role in the activation of the immune system. Polymorphisms within the genes encompassing the MHC class II structure influence the immune system by at least two mechanisms. First, polymorphic amino-acid residues on distinct class II proteins determine whether or not an individual peptide will bind and therefore be presented by a particular class II molecule displayed on an antigen-presenting cell. Second, MHC class II molecules regulate the developmental selection of T-cell receptor specificity in the thymus, thereby affecting the repertoire of $CD8^+$ and $CD4^+$ T-cells that recognise foreign peptides in the context of MHC class II molecules [117,140].

The first indication that treatment-resistant Lyme borreliosis might be an autoimmune disease came from a study analysing MHC II alleles (HLA-DR4) in patients with Lyme arthritis of brief, moderate or chronic duration [141]. Patients with chronic treatment-resistant Lyme arthritis have been found to have MHC II alleles that are also associated with rheumatoid arthritis, particularly HLA-DRB1* 0401 and 0101 alleles [142]. Most macrophages are able to internalise *B. burgdorferi* [143–147], and many investigators have demonstrated the localisation of intracellular *B. burgdorferi* within well-defined phagolysosomes [146,148,149] (Fig. 3). Filgueira *et al.* [150] showed that borrelial antigens were co-localised with MHC class Π molecules within lysosomal vesicles of dendritic cells. These studies suggest that *Borrelia* antigens are loaded on to MHC class Π molecules and are presented subsequently to $CD4^+$ T-lymphocytes.

It is of interest that synovial fibroblasts, which harbour *B. burgdorferi in vitro*, are able to present not only MHC I but also MHC II molecules [99,100]. Gross *et al.* [101] suggested that LFA-1 can serve as a cross-reactive autoantigen for OspA-reactive Th1 cells, leading to treatment-resistant Lyme arthritis. This was the first time that criteria for an autoimmune disease caused by molecular mimicry of microbial epitopes were

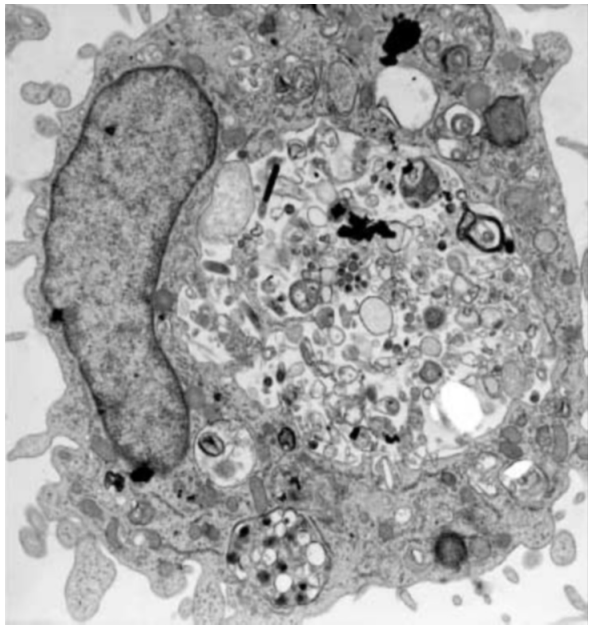

Fig. 3. Transmission electronmicrograph of a human peripheral blood monocyte, showing conventional phagocytosis of a *Borrelia burgdorferi* strain *sensu strictu* in a large central and smaller phagolysosome at the bottom of the figure.

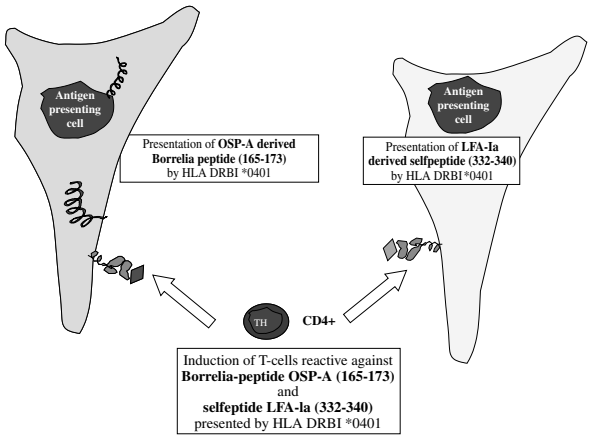

Fig. 4. Molecular mimicry in Lyme arthritis, depicting antigen-presenting cells (in this case monocytes, macrophages, dendritic cells and synovial fibroblasts) presenting peptides generated from borrelial OspA and host LFA-Ia (human leucocyte function-associated antigen 1), which induce a cross-reactive T-cell response.

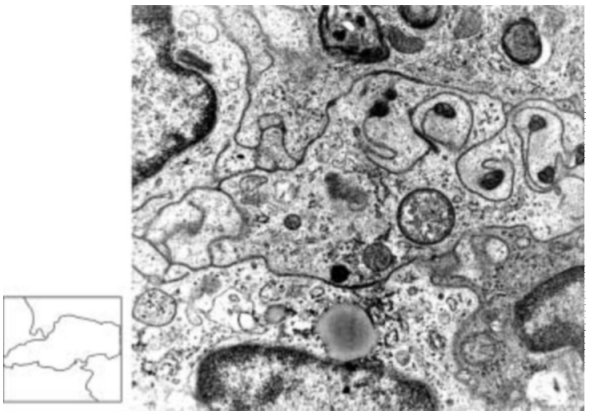

Fig. 5. Coiling phagocytosis of *Borrelia burgdorferi* by a cluster of human peripheral blood monocytes with their borders outlined in the scheme. The central cell shows multiple coiling phagocytosis figures probably trying to engulf one single spirochaete.

demonstrated in treatment-resistant Lyme arthritis [101,102,151–153]. Fig. 4 summarises these findings schematically, and suggests the possibility that intracellular persistence of *B. burgdorferi* in synovial cells might contribute to this scenario of molecular mimicry.

These studies were supported by the analysis of molecular mimicry in chronic neuroborreliosis. Hemmer *et al.* [154] demonstrated elegantly that several T-cell clones were responding to *Borrelia* peptides and endogenous host peptides. So far, these data on autoimmunity have been generated from adult Lyme disease patients. Comparable data on the pathogenesis of Lyme disease in children are pending.

Coiling phagocytosis has also been described as a mechanism for internalisation of *B. burgdorferi* [150,155–157] (Fig. 5). Coiling phagocytosis results in cytosolic deposition of antigens [150,156] and has been suggested as a mechanism for MHC class I presentation of exogenous antigens [158]. The presence of cytosolic *B. burgdorferi* has also been documented within human macrophages and granulocytes [156]. Likewise, an intracellular location of *B. burgdorferi* has been described for human dendritic cells [150], fibroblasts and synovial cells (Fig. 2) [100,159], endothelial cells [160] and murine marophages [146]. The ability of spirochaetes to enter the cytoplasm of macrophages may result in MHC class I presentation of *Borrelia* antigens to $CD4^{-}$ $CD8^{+}$ T-lymphocytes. In support of this, Busch *et al.* [116] demonstrated the existence of *B. burgdorferi*-specific $CD8^{+}$ T-lymphocytes in patients with Lyme borreliosis in remission. These studies suggest that *B. burgdorferi*-specific $CD8^{+}$ and $CD4^{+}$ T-lymphocytes can participate in the pathogenesis of Lyme arthritis.

DIAGNOSIS OF LYME DISEASE

The diagnosis of Lyme disease is based usually on recognition of the characteristic clinical features and a history of exposure in an area where the disease is endemic. For the diagnosis of an infectious disease, the standard is isolation of the causative agent in culture. Culture of *B. burgdorferi* from different specimens in Barbour–Stoenner–Kelly medium would permit a definitive diagnosis. However, positive cultures have generally only been obtained early in the course of disease, primarily from biopsy samples of erythema migrans lesions, less often from plasma samples [48], and only occasionally from CSF of patients with acute meningitis or facial paralysis [161]. At a later stage of the infection, PCR testing is greatly superior to culture for detection of *B. burgdorferi* in synovial fluid [162]. *B. burgdorferi* has not been isolated from the CSF of patients with chronic neuroborreliosis, but *B. burgdorferi* DNA has been detected in CSF from a limited number of these patients [163].

The serological tests used most commonly to diagnose *B. burgdorferi* infection include enzyme-linked immunosorbent assay, indirect immunofluorescence assay and Western blotting [164]. In

most cases, serological findings are dependent on the duration of the disease and clinical manifestations. In the early stages, < 50% of patients have detectable antibodies, predominantly IgM. In the late stages, seropositivity rises to 70–90%, with a shift from IgM to IgG antibodies. An antibody response to *B. burgdorferi* analysed by enzyme-linked immunosorbent assay and Western blotting, and interpreted according to the criteria of the Centers for Disease Control, is the current standard for diagnosis of Lyme disease [122]. In Europe, three different strains of *B. burgdorferi* contribute to the disease, in contrast to one strain in the USA. No single set of criteria for the interpretation of immunoblots results in a high level of sensitivity and specificity in all European countries [165], and antibiotic therapy may prevent an increase in specific antibodies. However, seroconversion may still occur after antibiotic therapy. Thus, if antibody titres are negative in early Lyme borreliosis, repetition of the tests has been recommended after *c.* 4 weeks [164]. In chronic disease, IgG antibody titres are usually high, and may remain so for several years, even after successful treatment. Rarely, patients with chronic disease remain seronegative, showing only a cell-mediated immune response to *B. burgdorferi* [166].

One possible cause of seronegativity is the formation of immune complexes by antigen-specific antibodies [167]. The great variability in serological test results between laboratories may be associated with differences in the test procedures used, different diagnostic cut-off values for the discrimination of positive or negative values, and different antigen preparations or strains of *B. burgdorferi* used to prepare the test. Western blotting has been recommended as a confirmatory test [168], but interpretation is often difficult because specific and cross-reactive bands can often appear close together. A promising approach to standardise Western blots is the use of a defined panel of recombinant antigens [169].

TREATMENT OF LYME DISEASE

Most patients treated for Lyme borreliosis have an excellent prognosis, although some patients treated for erythema migrans in recent series continue to have a variety of complaints after antibiotic therapy. One recent study in New England, USA, found that most Lyme borreliosis patients who were not feeling well 3 months after treatment had laboratory evidence of coinfection with *Babesia* spp. [170]. Patients with carditis and neurological disorders also tend to do well after treatment, although some adult patients have residual neurological deficits such as mild seventh cranial nerve palsy after treatment [171]. Oral doxycycline or intravenous ceftriaxone are usually effective in the treatment of Lyme arthritis, in combination with non-steroidal anti-inflammatory drugs [172]. If arthritis persists despite the completion of two courses of antibiotic therapy, intra-articular steroids, disease-modifying anti-rheumatic drugs or arthroscopic synovectomy may be introduced. After appropriate treatment of Lyme disease, a small percentage of adult patients continue to report subjective symptoms such as musculo-skeletal pain, neurocognitive difficulties, or fatigue that may last for years. This disabling syndrome has been termed post-Lyme disease syndrome. Clinically, it resembles chronic fatigue syndrome or fibromyalgia [173]. It occurs more frequently in adult patients with symptoms suggestive of early dissemination of the spirochaete into the nervous system, and particularly if treatment was delayed [174].

Treatment of Lyme disease in children is comparable to recommendations established for adult disease [175]. Doxycycline, however, should not be used in the treatment of children aged < 10 years. Early manifestations (except cardiac or neurological involvement) are treated with oral amoxycillin (50 mg/kg/day) or oral doxycycline (100 mg/day in two doses) for 14–21 days. Meningitis, meningoradiculitis, cardiac manifestations, Lyme arthritis and chronic treatment-resistant arthritis are treated with intravenous ceftriaxone 50–75 mg/kg/day once-daily for 14 days. Alternatively, intravenous cefotaxime (150–200 mg/kg/day) for 14 days, oral amoxycillin (50 mg/kg/day) or oral doxycycline (100–200 mg/day in two doses) for 30 days may be used [77].

PREVENTION OF LYME DISEASE

Avoid tick bites

Hikers can reduce exposure to ticks by walking wide trails. Preferred dress is light-coloured clothing (to make recognition and removal of the ticks easier), with long sleeves that are tight at the wrists and long trousers that are tucked into

light-coloured socks. A hat should be worn in densely wooded areas [176]. Habitats that are heavily infested with ticks, such as wooded areas, should be avoided if possible.

Tick and insect repellents that contain n,n-dimethylmetatoluamide applied to the skin provide additional protection, but require reapplication every 1–2 h for maximum effectiveness. Neurological complications in children from either frequent or excessive application of n, n-dimethylmetatoluamide-containing repellents have been reported. However, the health risk is low when these products are used according to the instructions [177]. n,n-Dimethylmetatoluamide should be applied sparingly only to exposed skin, and not to a child's face.

Permethrin (synthetic pyrethroid) is an insecticide derived from the chrysanthemum family of plants. It is used as a spray only on cloth, and it is deactivated on the skin. Once it is sprayed on to clothing, it becomes odourless. The effect of one single application can last for several weeks. Once it is applied, most ticks which come into contact with it will curl up, fall off, and eventually die.

Most studies show that transmission of *B. burgdorferi* from infected ticks usually requires a prolonged duration of attachment (12–48 h) to the host [178]. Therefore, attached ticks should be removed quickly with the help of medium-tipped tweezers as close to the skin as possible. If remnants of bite apparatus remain embedded in the skin, they should be left there. These are usually extruded parts, and additional attempts to remove them often result in unnecessary damage to tissues, which may increase the risk of local bacterial infection [136].

Eradication of ticks

In endemic residential areas, clearing bushes and trees, removing leaf litter and removing wood piles has been suggested. Pesticides have been used to suppress the tick population in residential areas. Erecting fences to exclude deer and ensuring that pets are tick-free may also reduce exposure to ticks [177].

Vaccines

Vaccines for Lyme disease that use recombinant OspA (rOspA) as an antigen have been produced by two manufacturers and field-tested for safety and efficacy in humans [136]. LYMErix (SmithKline Beecham Pharmaceuticals, Philadelphia, PA, USA) contains 30 mg of purified rOspA lipidated protein combined with 0.5 mg of aluminium adjuvant. This is the only licensed Lyme disease vaccine at this time, but marketing has been discontinued because of limited demand. Imulyme, a vaccine produced by Pasteur Merieux Connaught (Swiftwater, PA, USA), which contains 30 mg of purified rOspA lipidated protein without adjuvant, has not been propagated further.

rOspA vaccines have a unique mode of action. OspA is expressed by *B. burgdorferi* organisms that reside in the mid-gut of dormant ticks. *B. burgdorferi* subsequently down-regulates the expression in response to a blood meal and in preparation for skin invasion. Thus, ticks must become engorged with blood before they can transmit the organism. Patients who have not been exposed previously to *B. burgdorferi* have little antibody response to OspA (at least in the early stages of infection). When an immunised host is bitten by a tick infected with *B. burgdorferi*, protective OspA antibodies of the host are ingested by the tick. These antibodies then destroy *B. burgdorferi* in the gut of the tick and thus prevent transmission to the host.

In a large clinical trial, Steere *et al.* [179] found that the efficacy of LYMErix in preventing infection was 83% in the first year and 100% in the second year. In another large trial, Sigal *et al.* [180] found that the efficacy of the Imulyme vaccine in preventing symptomatic Lyme disease after the third injection was 92%. Some of the differences in the efficacy of the vaccines between the two studies may be explained by differences in methods of surveillance. The rOspA vaccines used in both clinical studies appeared to be safe. The vaccine-related side effects reported most frequently were pain, redness and swelling at the sites of injection [136]. These effects were usually mild and limited. The rOspA vaccine does not protect all recipients from infection with *B. burgdorferi* and provides no protection against other tick-borne diseases [136]. Therefore, vaccinated persons should also continue to take personal protective measures against tick bites.

ACKNOWLEDGEMENTS

The authors would like to thank J. Hacker, M. Frosch and K. Brehm for continuous support and reading the manuscript.

REFERENCES

1. Orloski KA, Hayes EB, Campbell GL, Dennis DT. Surveillance for Lyme disease—United States, 1992–1998. *MMWR CDC Surveill Summ* 2000; **49**: 1–11.
2. Stanek G. Lyme disease and related disorders. *Microbiol Sci* 1985; **2**: 231–234.
3. Fraser CM, Casjens S, Huang WM *et al*. Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. *Nature* 1997; **390**: 580–586.
4. de Silva AM, Fikrig E. Arthropod and host gene expression by *Borrelia burgdorferi*. *J Clin Invest* 1997; **99**: 377–379.
5. Anderson JF. Epizootiology of *Lyme borreliosis*. *Scand J Infect Dis* 1991; **77**(suppl): 23–34.
6. Piesman J. Standard system for infecting ticks (Acari: Ixodidae) with the Lyme disease spirochete, *Borrelia burgdorferi*. *J Med Entomol* 1993; **30**: 199–203.
7. Wilske B, Steinhuber R, Bergmeister H *et al*. Lyme borreliosis in South Germany. Epidemiologic data on the incidence of cases and on the epidemiology of ticks (Ixodes ricinus) carrying *Borrelia burgdorferi*. *Dtsch Med Wochenschr* 1987; **112**: 1730–1736.
8. Burgdorfer W, Anderson JF, Gern L, Lane RS, Piesman J, Spielman A. Relationship of *Borrelia burgdorferi* to its arthropod vectors. *Scand J Infect Dis* 1991; **77**: 35–40.
9. Aeschlimann A. Development embryonnaire d'Ornithodorus moubata et transmission transovarienne de *Borrelia duttoni*. *Acta Trop* 1958; **15**: 15–64.
10. Balashov YS. Transovarial transmission of spirochete *Borrelia sogdiana* in *Ornithodoros papillipes* ticks and its effect on the biological properties of the agent [in Russian]. *Parazitilogiya* 1968; **2**: 198–201.
11. Hayes SF, Burgdorfer W, Barbour AG. Bacteriophage in the *Ixodes dammini* spirochete, etiological agent of Lyme disease. *J Bacteriol* 1983; **154**: 1436–1439.
12. Hayes SF, Burgdorfer W. Ultrastructure of *Borrelia burgdorferi*. In: Weber K, Burgdorfer W, eds. *Aspects of Lyme borreliosis*. Berlin: Springer, 1993; 29–43.
13. Hans-Walter P, Bettina W, Weber K. Lyme borreliosis: basic science and clinical aspects. *Lancet* 1994; **343**: 1013–1016.
14. Norris SJ, Carter CJ, Howell JK, Barbour AG. Low-passage-associated proteins of *Borrelia burgdorferi* B31: characterization and molecular cloning of OspD, a surface-exposed, plasmid-encoded lipoprotein. *Infect Immun* 1992; **60**: 4662–4672.
15. Lam TT, Nguyen TP, Montgomery RR, Kantor FS, Fikrig E, Flavell RA. Outer surface proteins E and F of *Borrelia burgdorferi*, the agent of Lyme disease. *Infect Immun* 1994; **62**: 290–298.
16. Umemoto T, Namikawa I. Electron microscopy of the spherical body of oral spirochetes in vitro. Further studies. *Microbiol Immunol* 1980; **24**: 321–334.
17. Barbour AG, Hayes SF. Biology of *Borrelia* species. *Microbiol Rev* 1986; **50**: 381–400.
18. Kersten A, Poitscheck S, Rauch S, Aberer E. Effects of penicillin, ceftriaxone and doxycycline on morphology of *Borrelia burgdorferi*. *Antimicrob Agents Chemother* 1995; **39**: 1127–1133.
19. Preac Mursic V, Weber K, Pfister HW *et al*. Formation and cultivation of *Borrelia burgdorferi* spheroplast 1-form variants. *Infection* 1989; **17**: 355–359.
20. Garon CF, Dorward DW, Corwin MD. Structural features of *Borrelia burgdorferi*—the Lyme disease spirochete: silver staining for nucleic acids. *Scanning Microsc* 1989; **3**(suppl): 109–115.
21. Alban SP, Paul W, Johnson PW, Nelson DR. Serum starvation induced changes in protein synthesis and morphology of *Borrelia burgdorferi*. *Microbiology* 2000; **146**: 119–127.
22. Brorson O, Brorson SH. In vitro conversion of *Borrelia burgdorferi* to cystic forms in spinal fluid, and transformation to mobile spirochetes by incubation in BSK-H media. *Infection* 1998; **26**: 144–150.
23. Aberer E, Koszik F, Silberer M. Why is chronic Lyme borreliosis chronic? *Clin Infect Dis* 1997; **25**: S64–S70.
24. Hulinska D, Bartak P, Hercogova J, Hancil J, Basta J, Schramlova J. Electron microscopy of Langerhans cells and *Borrelia burgdorferi* in Lyme disease patients. *Zentralbl Bakteriol* 1994; **280**: 348–359.
25. Holt SC. Anatomy and chemistry of spirochetes. *Microbiol Rev* 1978; **42**: 114–160.
26. Hovind-Hougen K. *Treponema* and *Borrelia* morphology. In: Johnson RC, ed. *The biology of parasitic spirochetes*. New York: Academic Press, 1976; 7–28.
27. Lofgren R, Soule MH. The structure of *Spirochaetea novyi* as revealed by electron microscopy. *Bacteriology* 1945; **50**: 679–690.
28. Hovind-Hougen K. Ultrastructure of spirochetes isolated from *Ixodes ricinus* and *Ixodes dammini*. *Yale J Biol Med* 1984; **57**: 543–548.
29. Hovind-Hougen K. Determination by means of electron microscopy of morphologic criteria of value for clarification of some spirochetes in particular treponemes. *Acta Pathol Mirobiol Scand* 1976; **255**(suppl B): 1–41.
30. Barbour G, Hayes SF, Heiland RA, Schrumpf ME, Tessier SL. A *Borrelia* genus specific monoclonal antibody binds to a flagellar epitope. *Infect Immun* 1986; **52**: 549–554.
31. Ginger CD. Isolation and characterization of muramic acid from two spirochetes: *Borrelia duttoni and Leptospira biflexa*. *Nature* 1963; **199**: 159.
32. Klaviter EC, Johnson RC. Isolation of the outer envelope, chemical components, and ultrastructure of *Borrelia hermsi* grown *in vitro*. *Acta Trop* 1979; **36**: 123–131.
33. Barbour AG, Schrumpf ME. Polymorphism of the major surface proteins of *Borrelia burgdorferi*. *Zentrabl Bakteriol Hyg* 1986; **263**: 83–91.
34. Schaible UE, Kramer MD, Eichmann K, Modolell M, Museteanu C, Simon MM. Monoclonal antibodies specific for the outer surface protein (OspA) prevent *Lyme borreliosis* in severe combined immunodeficiency (SCID). *Proc Natl Acad Sci USA* 1990; **87**: 3768–3772.
35. Preac-Mursic V, Wilske B, Patsouris E *et al*. Active immunization with pC protein of *Borrelia burgdorferi* protects gerbils against *B. burgdorferi* infection. *Infection* 1992; **20**: 342–349.
36. Burkot TR, Piesman J, Wirt RA. Quantitation of *Borrelia burgdorferi* outer surface protein A in *Ixodes scapularis*. Fluctuations during the tick life cycle, doubling times and loss while feeding. *J Infect Dis* 1994; **170**: 883–889.
37. Schwan TG, Piesman J, Golde WT, Dolan MC, Rosa PA. Induction of an outer surface protein on *Borrelia burgdorferi* during tick feeding. *Proc Natl Acad Sci USA* 1995; **92**: 2909–2913.

38. Montgomery RR, Malawista SE, Feen KJ, Bockenstedt LK. Direct demonstration of antigenic substitution of *Borrelia burgdorferi* ex vivo: exploration of the paradox of the early immune response to outer surface proteins A and C in Lyme disease. *J Exp Med* 1996; **183**: 261–269.
39. Obonyo M, Munderloh UG, Fingerle V, Wilske B, Kurtti TJ. *Borrelia burgdorferi* in tick cell culture modulates expression of outer surface proteins A and C in response to temperature. *J Clin Microbiol* 1999; **37**: 2137–2141.
40. Stevenson B, Schwan TG, Rosa PA. Temperature-related differential expression of antigens in the Lyme disease spirochete, *Borrelia burgdorferi*. *Infect Immun* 1995; **63**: 4535–4539.
41. Stevenson B, Tilly K, Rosa PA. A family of genes located on four separate 32-kilobase circular plasmids in *Borrelia burgdorferi* B31. *J Bacteriol* 1996; **178**: 3508–3516.
42. Zhang JR, Norris SJ. Genetic variation of the *Borrelia burgdorferi* gene *vlse* involves cassette specific, segmental gene conversion. *Infect Immun* 1998; **66**: 3698–3704.
43. Steere AC. Lyme disease. *N Engl J Med* 1989; **321**: 586–596.
44. Weber K, Pfister HW, Reimers CD. Clinical features of Lyme borreliosis: clinical overview. In: Weber K, Burgdorfer W, eds. *Aspects of Lyme borreliosis*. Berlin: Springer, 1993; 93–104.
45. Hengge UR, Tannapfel A, Tyring SK, Erbel R, Arendt G, Ruzicka T. Lyme borreliosis. *Lancet Infect Dis* 2003; **3**: 489–500.
46. Wahlberg P, Granlund H, Nyman D, Panelius J, Seppala I. Late Lyme borreliosis: epidemiology, diagnosis and clinical features. *Ann Med* 1993; **25**: 349–352.
47. Strle F, Nadelman RB, Cimperman J *et al.* Comparison of culture-confirmed erythema migrans caused by *Borrelia burgdorferi* sensu stricto in New York State and by *Borrelia afzelii* in Slovenia. *Ann Intern Med* 1999; **130**: 32–36.
48. Wormser GP, Bittker S, Cooper D, Nowakowski J, Nadelman RB, Pavia C. Comparison of the yields of blood cultures using serum or plasma from patients with early Lyme disease. *J Clin Microbiol* 2000; **38**: 1648–1650.
49. Stanek G, O'Connell S, Cimmino M *et al.* European Union Concerted Action on Risk Assessment in Lyme Borreliosis: clinical case definitions for Lyme borreliosis. *Wien Klin Wochenschr* 1996; **108**: 741–747.
50. Picken RN, Strle F, Ruzic-Sabljic E *et al.* Molecular subtyping of *Borrelia burgdorferi sensu lato* isolates from five patients with solitary lymphocytoma. *J Invest Dermatol* 1997; **108**: 92–97.
51. Roberts ED, Bohm RP, Lowrie RC *et al.* Pathogenesis of Lyme neuroborreliosis in the rhesus monkey: the early disseminated and chronic phases of disease in the peripheral nervous system. *J Infect Dis* 1998; **178**: 722–732.
52. Cadavid D, O'Neill T, Schaefer H, Pachner AR. Localization of *Borrelia burgdorferi* in the nervous system and other organs in a nonhuman primate model of lyme disease. *Lab Invest* 2000; **80**: 1043–1054.
53. Stanek G, Klein J, Bittner R, Glogar D. Isolation of *Borrelia burgdorferi* from the myocardium of a patient with longstanding cardiomyopathy. *N Engl J Med* 1990; **322**: 249–252.
54. Steere AC, Duray PH, Butcher EC. Spirochetal antigens and lymphoid cell surface markers in Lyme synovitis. Comparison with rheumatoid synovium and tonsillar lymphoid tissue. *Arthritis Rheum* 1988; **31**: 487–495.
55. Rothermel H, Hedges TR, Steere AC. Optic neuropathy in children with Lyme disease. *Pediatrics* 2001; **108**: 477–481.
56. Oschmann P, Dorndorf W, Hornig C, Schafer C, Wellensiek HJ, Pflughaupt KW. Stages and syndromes of neuroborreliosis. *J Neurol* 1998; **245**: 262–272.
57. Logigian EL, Steere AC. Clinical and electrophysiologic findings in chronic neuropathy of Lyme disease. *Neurology* 1992; **42**: 303–311.
58. Logigian EL, Kaplan RF, Steere AC. Chronic neurologic manifestations of Lyme disease. *N Engl J Med* 1990; **323**: 1438–1444.
59. Halperin JJ, Luft BJ, Anand AK *et al.* Lyme neuroborreliosis: central nervous system manifestations. *Neurology* 1989; **39**: 753–759.
60. Halperin JJ. Lyme disease and the peripheral nervous system. *Muscle Nerve* 2003; **28**: 133–143.
61. Logigian EL, Kaplan RF, Steere AC. Successful treatment of Lyme encephalopathy with intravenous ceftriaxone. *J Infect Dis* 1999; **180**: 377–383.
62. Gerber MA, Shapiro ED, Burke GS, Parcells VJ, Bell GL. Lyme disease in children in southeastern Connecticut. Pediatric Lyme Disease Study Group. *N Engl J Med* 1996; **335**: 1270–1274.
63. Strobino BA, Williams CL, Abid S, Chalson R, Spierling P. Lyme disease and pregnancy outcome: a prospective study of two thousand prenatal patients. *Am J Obstet Gynecol* 1993; **169**: 367–374.
64. Nadal D, Hunziker UA, Bucher HU, Hitzig WH, Duc G. Infants born to mothers with antibodies against *Borrelia burgdorferi* at delivery. *Eur J Pediatr* 1989; **148**: 426–427.
65. Williams CL, Strobino B, Weinstein A, Spierling P, Medici F. Maternal Lyme disease and congenital malformations: a cord blood serosurvey in endemic and control areas. *Paediatr Perinat Epidemiol* 1995; **9**: 320–330.
66. Strobino B, Abid S, Gewitz M. Maternal Lyme disease and congenital heart disease: a case-control study in an endemic area. *Am J Obstet Gynecol* 1999; **180**: 711–716.
67. Elliott DJ, Eppes SC, Klein JD. Teratogen update: Lyme disease. *Teratology* 2001; **64**: 276–281.
68. Culp RW, Eichenfield AH, Davidson RS, Drummond DS, Christofersen MR, Goldsmith DP. Lyme arthritis in children. An orthopaedic perspective. *J Bone Joint Surg Am* 1987; **69**: 96–99.
69. Eichenfield AH, Goldsmith DP, Benach JL *et al.* Childhood Lyme arthritis: experience in an endemic area. *J Pediatr* 1986; **109**: 753–758.
70. Eppes SC, Nelson DK, Lewis LL, Klein JD. Characterization of Lyme meningitis and comparison with viral meningitis in children. *Pediatrics* 1999; **103**: 957–960.
71. Huppertz HI, Munchmeier D, Lieb W. Ocular manifestations in children and adolescents with Lyme arthritis. *Br J Ophthalmol* 1999; **83**: 1149–1152.
72. Lesser RL, Kornmehl EW, Pachner AR *et al.* Neuro-ophthalmologic manifestations of Lyme disease. *Ophthalmology* 1990; **97**: 699–706.
73. Farris BK, Webb RM. Lyme disease and optic neuritis. *J Clin Neuroopthalmol* 1988; **8**: 73–78.
74. Scott IU, Silva-Lepe A, Siatkowski RM. Chiasmal optic neuritis in Lyme disease. *Am J Ophthalmol* 1997; **123**: 136–138.
75. Winward KE, Smith JL. Ocular disease in Caribbean patients with serologic evidence of Lyme borreliosis. *J Clin Neuroophthalmol* 1989; **9**: 65–70.

76. Bentas W, Karch H, Huppertz HI. Lyme arthritis in children and adolescents: outcome 12 months after initiation of antibiotic therapy. *J Rheumatol* 2000; **27**: 2025–2030.
77. Huppertz HI. Lyme disease in children. *Curr Opin Rheumatol* 2001; **13**: 434–440.
78. Steere AC, Schoen RT, Taylor E. The clinical evolution of Lyme arthritis. *Ann Intern Med* 1987; **107**: 725–731.
79. Zemel LS. Lyme disease—a pediatric perspective. *J Rheumatol* 1992; **34**(suppl): 1–13.
80. Fraser CM, Norris SJ, Weinstock GM *et al*. Complete genome sequence of *Treponema pallidum*, the syphilis spirochete. *Science* 1998; **281**: 375–388.
81. Bunkis J, Barbour AG. Access of antibody or trypsin to an integral outer membrane protein (P66) of *Borrelia burgdorferi* is hindered by Osp lipoproteins. *Infect Immun* 1999; **67**: 2874–2883.
82. Sadziene A, Barbour AG. Growth inhibition of *Borrelia burgdorferi sensu lato* by antibodies. A contribution to understanding the pathogenesis and improving diagnosis of Lyme borreliosis. *Wien Med Wochenschr* 1995; **145**: 162–165.
83. Seinost G, Dykhuizen DE, Dattwyler RJ *et al*. Four clones of *Borrelia burgdorferi sensu stricto* cause invasive infection in humans. *Infect Immun* 1999; **67**: 3518–3524.
84. Coleman JL, Gebbia JA, Piesman J, Degen JL, Bugge TH, Benach JL. Plasminogen is required for efficient dissemination of *B. burgdorferi* in ticks and for enhancement of spirochetemia in mice. *Cell* 1997; **89**: 1111–1119.
85. Coburn J, Magoun L, Bodary SC, Leong JM. Integrins alpha(v)beta3 and alpha5beta1 mediate attachment of Lyme disease spirochetes to human cells. *Infect Immun* 1998; **66**: 1946–1952.
86. Guo BP, Brown EL, Dorward DW, Rosenberg LC, Hook M. Decorin binding adhesions from *Borrelia burgdorferi*. *Mol Microbiol* 1998; **30**: 711–723.
87. Probert WS, Johnson BJ. Identification of a 47 kDa fibronectin-binding protein expressed by *Borrelia burgdorferi* isolate B31. *Mol Microbiol* 1998; **30**: 1003–1015.
88. Guo BP, Norris SJ, Rosenberg LC, Hook M. Adherence of *Borrelia burgdorferi* to proteoglycan decorin. *Infect Immun* 1995; **63**: 3467–3472.
89. Feng S, Hodzic E, Stevenson B, Barthold SW. Humoral immunity to *Borrelia burgdorferi* N40 decorin binding proteins during infection of laboratory mice. *Infect Immun* 1998; **66**: 2827–2835.
90. Skare JT, Foley DM, Hernandez SR *et al*. Cloning and molecular characterization of plasmid-encoded antigens of *Borrelia burgdorferi*. *Infect Immun* 1999; **67**: 4407–4417.
91. Hagman KE, Lahdenne P, Popova TG *et al*. Decorin-binding protein of *Borrelia burgdorferi* is encoded within a two-gene operon and is protective in the murine model of Lyme borreliosis. *Infect Immun* 1998; **66**: 2674–2683.
92. Brown EL, Wooten RM, Johnson BJ *et al*. Resistance to Lyme disease in decorin-deficient mice. *J Clin Invest* 2001; **107**: 845–852.
93. Silberer M, Koszik F, Stingl G, Aberer E. Downregulation of class II molecules on epidermal Langerhans cells in Lyme borreliosis. *Br J Dermatol* 2000; **143**: 786–794.
94. Sellati TJ, Burns MJ, Ficazzola MA, Furie MB. *Borrelia burgdorferi* upregulates expression of adhesion molecules on endothelial cells and promotes transendothelial migration of neutrophils in vitro. *Infect Immun* 1995; **63**: 4439–4447.
95. Leong JM, Morrissey PE, Ortega-Barria E, Pereira ME, Coburn J. Hemagglutination and proteoglycan binding by the Lyme disease spirochete, *Borrelia burgdorferi*. *Infect Immun* 1995; **63**: 874–883.
96. Kopp PA, Schmitt M, Wellensiek HJ, Blobel H. Isolation and characterization of fibronectin-binding sites of *Borrelia garinii* N34. *Infect Immun* 1995; **63**: 3804–3808.
97. Guner ES. Complement evasion by the Lyme disease spirochete *Borrelia burgdorferi* grown in host-derived tissue co-cultures: role of fibronectin in complement-resistance. *Experientia* 1996; **52**: 364–372.
98. Comstock L, Thomas DD. Characterization of *Borrelia burgdorferi* invasion of cultured endothelial cells. *Microb Pathog* 1991; **10**: 137–148.
99. Girschick HJ, Meister S, Karch H, Huppertz HI. *Borrelia burgdorferi* downregulates ICAM-1 on human synovial cells in vitro. *Cell Adhes Commun* 1999; **7**: 73–83.
100. Girschick HJ, Huppertz HI, Russmann H, Krenn V, Karch H. Intracellular persistence of *Borrelia burgdorferi* in human synovial cells. *Rheumatol Int* 1996; **16**: 125–132.
101. Gross DM, Forsthuber T, Tary-Lehmann M *et al*. Identification of LFA-1 as a candidate autoantigen in treatment-resistant Lyme arthritis. *Science* 1998; **281**: 703–706.
102. Steere AC, Gross D, Meyer AL, Huber BT. Autoimmune mechanisms in antibiotic treatment-resistant Lyme arthritis. *J Autoimmun* 2001; **16**: 263–268.
103. Krause A, Brade V, Schoerner C, Solbach W, Kalden JR, Burmester GR. T cell proliferation induced by *Borrelia burgdorferi* in patients with Lyme borreliosis. Autologous serum required for optimal stimulation. *Arthritis Rheum* 1991; **34**: 393–402.
104. Neumann A, Schlesier M, Schneider H, Vogt A, Peter HH. Frequencies of *Borrelia burgdorferi*-reactive T lymphocytes in Lyme arthritis. *Rheumatol Int* 1989; **9**: 237–241.
105. Roessner K, Fikrig E, Russel JQ, Cooper SM, Favell RA, Budd RC. Prominent T lymphocyte response to *Borrelia burgdorferi* from peripheral blood of unexposed donors. *Eur J Immunol* 1994; **24**: 320–324.
106. Wallach FR, Murray HW. Lymphocyte proliferation assay in Lyme disease. *J Infect Dis* 1992; **166**: 938–939.
107. Zoschke DC, Skemp AA, Defosse DL. Lymphoproliferative responses to *Borrelia burgdorferi* in Lyme disease. *Ann Intern Med* 1991; **114**: 285–289.
108. Huppertz HI, Mosbauer S, Busch DH, Karch H. Lymphoproliferative responses to *Borrelia burgdorferi* in the diagnosis of Lyme arthritis in children and adolescents. *Eur J Pediatr* 1996; **155**: 297–302.
109. Rutkowski S, Busch DH, Huppertz HI. Lymphocyte proliferation assay in response to *Borrelia burgdorferi* in patients with Lyme arthritis: analysis of lymphocyte subsets. *Rheumatol Int* 1997; **17**: 151–158.
110. Lengl-Janssen B, Strauss AF, Steere AC, Kamradt T. The T helper cell response in Lyme arthritis: differential recognition of *Borrelia burgdorferi* outer surface protein A in patients with treatment-resistant or treatment-responsive Lyme arthritis. *J Exp Med* 1994; **180**: 2069–2078.
111. Yoshinari NH, Reinhardt BN, Steere AC. T cell responses to polypeptide fractions of *Borrelia burgdorferi* in patients with Lyme arthritis. *Arthritis Rheum* 1991; **34**: 707–713.
112. Yssel H, Nakamoto T, Schneider P *et al*. Analysis of T lymphocytes cloned from synovial fluid and blood of a

patient with Lyme arthritis. *Int Immunol* 1990; **2**: 1081–1089.
113. Yssel H, Shanafelt MC, Soderberg C, Schneider PV, Anzola J, Pelty G. *Borrelia burgdorferi* activates a T helper type 1-like cell subset in Lyme arthritis. *J Exp Med* 1991; **174**: 593–601.
114. Lim LC, England DM, DuChateau BK, Glowacki NJ, Schell RF. *Borrelia burgdorferi*-specific T lymphocytes induce severe destructive Lyme arthritis. *Infect Immun* 1995; **63**: 1400–1408.
115. Matyniak JE, Reiner SL. T helper phenotype and genetic susceptibility in experimental Lyme disease. *J Exp Med* 1995; **181**: 1251–1254.
116. Busch DH, Jassoy C, Brinckmann U, Girschick H, Huppertz HI. Detection of *Borrelia burgdorferi*-specific CD8+ cytotoxic T cells in patients with Lyme arthritis. *J Immunol* 1996; **157**: 3534–3541.
117. Gross DM, Huber BT. Cellular and molecular aspects of Lyme arthritis. *Cell Mol Life Sci* 2000; **57**: 1562–1569.
118. de Souza MS, Smith AL, Beck DS, Terwilliger GA, Fikrig E, Barthold SW. Long term study of cell mediated responses to *Borrelia burgdorferi* in the laboratory mouse. *Infect Immun* 1993; **61**: 1814–1822.
119. Dressler F, Whalen JA, Reinhardt BN, Steere AC. Western blotting in the serodiagnosis of Lyme disease. *J Infect Dis* 1993; **167**: 392–400.
120. Huppertz HI, Bohme M, Standaert SM, Karch H, Plotkin SA. Incidence of Lyme borreliosis in the Wurzburg region of Germany. *Eur J Clin Microbiol Infect Dis* 1999; **18**: 697–703.
121. Hobusch D, Christen HJ, Huppertz HI, Noack R. Diagnosis and therapy of Lyme borreliosis in children. Practice guideline of the German Society for Pediatric Infectious Diseases. *Klin Padiatr* 1999; **211**: 70–74.
122. Steere AC. Lyme disease. *N Engl J Med* 2001; **345**: 115–125.
123. Huppertz HI, Karch H, Suschke HJ *et al*. Lyme arthritis in European children and adolescents. The Pediatric Rheumatology Collaborative Group. *Arthritis Rheum* 1995; **38**: 361–368.
124. Paul WE. *Fundamental immunology*. New York: Raven Press, 1993.
125. Du Chateau BK, England DM, Callister SM, Lim LC, Lovrich SD, Schell RF. Macrophages exposed to *Borrelia burgdorferi* induce Lyme arthritis in hamsters. *Infect Immun* 1996; **64**: 2540–2547.
126. DuChateau BK, Jensen JR, England DM, Callister SM, Lovrich SD, Schell RF. Macrophages and enriched populations of T lymphocytes interact synergistically for the induction of severe, destructive Lyme arthritis. *Infect Immun* 1997; **65**: 2829–2836.
127. Beck G, Benach JL, Habicht GS. Isolation of interleukin 1 from joint fluids of patients with Lyme disease. *J Rheumatol* 1989; **16**: 800–806.
128. De-Fosse DL, Johnson RC. In-vitro and in-vivo induction of tumor necrosis factor alpha by *Borrelia burgdorferi*. *Infect Immunol* 1992; **60**: 1109–1113.
129. Miller LC, Lynch EA, Isa S, Logan JW, Dinareloo CA, Steere AC. Balance of synovial fluid interleukin-1beta and interleukin-1 receptor antagonist and recovery from Lyme arthritis. *Lancet* 1993; **341**: 146–148.
130. Steere AC, Brinckerhoff CE, Miller DJ, Drinker H, Harris AD, Malaista SE. Elevated levels of collagenase and prostaglandin E2 from synovium associated with erosion of cartilage and bone in a patient with chronic Lyme arthritis. *Arthritis Rheum* 1980; **23**: 591–599.
131. Bertolini DR, Nedwin GE, Bringman TS, Smith DD, Mundy GR. Stimulation of bone resorption and inhibition of bone formation *in-vitro* by human tumor necrosis factors. *Nature* 1986; **319**: 516–518.
132. Ma Y, Seiler KP, Tai K, Yang L, Woods M, Weis JJ. Outer surface lipoproteins of *Borrelia burgdorferi* stimulate nitric oxide production by the cytokine inducible pathway. *Infect Immun* 1994; **62**: 3663–3671.
133. McCartney-Francis N, Allen JB, Mizel DE *et al*. Suppression of arthritis by an inhibitor of nitric oxide synthase. *J Exp Med* 1993; **178**: 749–754.
134. Weinberg JB, Granger DL, Pisetsky DS *et al*. The role of nitric oxide in the pathogenesis of spontaneous murine autoimmune disease: increased nitric oxide production and nitric oxide synthase expression in MRL-lpr/lpr mice, and reduction of spontaneous glomerulonephritis and arthritis by orally administered NG-monomethyl-L-arginine. *J Exp Med* 1994; **179**: 651–660.
135. Gajdusek DC. Hypothesis: interference with axonal transport of neurofilament as a common pathogenetic mechanism in certain diseases of the central nervous system. *N Engl J Med* 1985; **312**: 714–719.
136. Shapiro ED, Gerber MA. Lyme disease. *Clin Infect Dis* 2000; **31**: 533–542.
137. Steere AC, Dwyer E, Winchester R. Association of chronic Lyme arthritis with HLA-DR4 and HLA-DR2 alleles. *N Engl J Med* 1990; **323**: 219–223.
138. Kalish RA, Leong JM, Steere AC. Early and late antibody responses to full-length and truncated constructs of outer surface protein A of *Borrelia burgdorferi* in Lyme disease. *Infect Immun* 1995; **63**: 2228–2235.
139. Kamradt T, Krause A, Burmester GR. A role for T cells in the pathogenesis of treatment-resistant Lyme arthritis. *Mol Med* 1995; **1**: 486–490.
140. Nepom GT, Erlich H. MHC class-II molecules and autoimmunity. *Ann Rev Immunol* 1991; **9**: 493–525.
141. Kalish RA, Leong JM, Steere AC. Association of treatment-resistant chronic Lyme arthritis with HLA-DR4 and antibody reactivity to OspA and OspB of *Borrelia burgdorferi*. *Infect Immun* 1993; **61**: 2774–2779.
142. Steere AC, Baxter-Lowe LA. Association of chronic, treatment resistant Lyme arthritis with rheumatoid arthritis (RA) alleles. *Arthritis Rheum* 1998; **41**: S81.
143. Benach JL, Fleit HB, Habicht GS, Coleman JL, Bosler EM, Lane BP. Interactions of phagocytes with the Lyme disease spirochete: role of the Fc receptor. *J Infect Dis* 1984; **150**: 497–507.
144. Benach JL, Habicht GS, Gocinski BL, Coleman JL. Phagocytic cell responses to in vivo and in vitro exposure to the Lyme disease spirochete. *Yale J Biol Med* 1984; **57**: 599–605.
145. Georgilis K, Noring R, Steere AC, Klempner MS. Neutrophil chemotactic factors in synovial fluids of patients with Lyme disease. *Arthritis Rheum* 1991; **34**: 770–775.
146. Montgomery RR, Nathanson MH, Malawista SE. The fate of *Borrelia burgdorferi*, the agent for Lyme disease, in mouse macrophages. Destruction, survival, recovery. *J Immunol* 1993; **150**: 909–915.
147. Peterson PK, Clawson CC, Lee DA, Garlich DJ, Quie PG, Johnson RC. Human phagocyte interactions with the Lyme disease spirochete. *Infect Immun* 1984; **46**: 608–611.

148. Montgomery RR, Malawista SE. Entry of *Borrelia burgdorferi* into macrophages is end-on and leads to degradation in lysosomes. *Infect Immun* 1996; **64**: 2867–2872.
149. Hechemy KE, Samsonoff WA, Harris HL, McKee M. Adherence and entry of *Borrelia burgdorferi* in Vero cells. *J Med Microbiol* 1992; **36**: 229–238.
150. Filgueiria L, Nestle FO, Rittig M, Joller HI, Groscurth P. Human dendritic cells phagocytose and process *Borrelia burgdorferi*. *J Immunol* 1996; **157**: 2998–3005.
151. Gross D, Huber BT, Steere AC. Molecular mimicry and Lyme arthritis. *Curr Dir Autoimmun* 2001; **3**: 94–111.
152. Klempner MS, Huber BT. Is it thee or me?—autoimmunity in Lyme disease. *Nat Med* 1999; **5**: 1346–1347.
153. Trollmo C, Meyer AL, Steere AC, Hafler DA, Huber BT. Molecular mimicry in Lyme arthritis demonstrated at the single cell level: LFA-1 alpha L is a partial agonist for outer surface protein A-reactive T cells. *J Immunol* 2001; **166**: 5286–5291.
154. Hemmer B, Gran B, Zhao Y *et al.* Identification of candidate T-cell epitopes and molecular mimics in chronic Lyme disease. *Nat Med* 1999; **5**: 1375–1382.
155. Modolell M, Schaible UE, Rittig M, Simon MM. Killing of *Borrelia burgdorferi* by macrophages is dependent on oxygen radicals and nitric oxide and can be enhanced by antibodies to outer surface proteins of the spirochete. *Immunol Lett* 1994; **40**: 139–146.
156. Rittig MG, Krause A, Haupl T *et al.* Coiling phagocytosis is the preferential phagocytic mechanism for *Borrelia burgdorferi*. *Infect Immun* 1992; **60**: 4205–4212.
157. Szczepanski A, Fleit HB. Interaction between *Borrelia burgdorferi* and polymorphonuclear leukocytes. Phagocytosis and the induction of respiratory burst. *Ann NY Acad Sci* 1988; **539**: 425–428.
158. Rittig MG, Haupl T, Burmester GR. Coiling phagocytosis: a way for MHC class I presentation of bacterial antigens? *Int Arch Allergy Immunol* 1994; **103**: 4–10.
159. Klempner MS, Noring R, Rogers RA. Invasion of human skin fibroblasts by the Lyme disease spirochete, *Borrelia burgdorferi*. *J Infect Dis* 1993; **167**: 1074–1081.
160. Ma Y, Sturrock A, Weis JJ. Intercellular localization of *Borrelia burgdorferi* within human endothelial cell. *Infect Immunol* 1991; **59**: 671–678.
161. Coyle PK, Goodman JL, Krupp LB, Loggiau EL, Reik L. *Lyme disease: continuum: lifelong learning in neurology*, Vol. 5. Philadelphia: Lippincott Williams & Wilkins, 1999.
162. Nocton JJ, Dressler F, Rutledge BJ, Rys PN, Persing DH, Steere AC. Detection of *Borrelia burgdorferi* by polymerase chain reaction in synovial fluid from patients with Lyme arthritis. *N Engl J Med* 1994; **330**: 229–234.
163. Nocton JJ, Bloom BJ, Rutledge BJ *et al.* Detection of *Borrelia burgdorferi* DNA by polymerase chain reaction in cerebrospinal fluid in Lyme neuroborreliosis. *J Infect Dis* 1996; **174**: 623–627.
164. Wilske B, Preac-Mursic V. Microbial diagnosis of Lyme borreliosis. In: Weber K, Burgdorfer W, eds. *Aspects of Lyme borreliosis*. Berlin: Springer, 1993; 267–300.
165. Robertson J, Guy E, Andrews N *et al.* A European multicenter study of immunoblotting in serodiagnosis of Lyme borreliosis. *J Clin Microbiol* 2000; **38**: 2097–2102.
166. Wilske B, Habermann C, Fingerle V *et al.* An improved recombinant IgG immunoblot for serodiagnosis of Lyme borreliosis. *Med Microbiol Immunol (Berl)* 1999; **188**: 139–144.
167. Schutzer SE, Coyle PK, Reid P, Holland B. *Borrelia burgdorferi*-specific immune complexes in acute Lyme disease. *JAMA* 1999; **282**: 1942–1946.
168. Grodzicki RL, Steere AC. Comparison of immunoblotting and indirect enzyme-linked immunosorbent assay using different antigen preparations for diagnosing early Lyme disease. *J Infect Dis* 1988; **157**: 790–797.
169. Lahdenne P, Panelius J, Saxen H *et al.* Improved serodiagnosis of erythema migrans using novel recombinant borrelial BBK32 antigens. *J Med Microbiol* 2003; **52**: 563–567.
170. Krause PJ, Telford SR, Spielman A *et al.* Concurrent Lyme disease and babesiosis. Evidence for increased severity and duration of illness. *JAMA* 1996; **275**: 1657–1660.
171. Halperin JJ, Logigian EL, Finkel MF, Pearl RA. Practice parameters for the diagnosis of patients with nervous system Lyme borreliosis (Lyme disease). Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* 1996; **46**: 619–627.
172. Dattwyler RJ, Halperin JJ, Volkman DJ, Luft BJ. Treatment of late Lyme borreliosis—randomised comparison of ceftriaxone and penicillin. *Lancet* 1988; **1**: 1191–1194.
173. Dinerman H, Steere AC. Lyme disease associated with fibromyalgia. *Ann Intern Med* 1992; **117**: 281–285.
174. Kalish RA, Kaplan RF, Taylor E, Jones-Woodward L, Workman K, Steere AC. Evaluation of study patients with Lyme disease, 10–20-year follow-up. *J Infect Dis* 2001; **183**: 453–460.
175. Wormser GP, Nadelman RB, Dattwyler RJ *et al.* Practice guidelines for the treatment of Lyme disease. The Infectious Diseases Society of America. *Clin Infect Dis* 2000; **31**(suppl 1): 1–14.
176. Shapiro ED. Lyme disease. *Pediatr Rev* 1998; **19**: 147–154.
177. Brown M, Hebert AA. Insect repellent: an overview. *J Am Acad Dermatol* 1997; **36**: 243–249.
178. Piesman J, Oliver JR, Sinsky RJ. Growth kinetics of the Lyme disease spirochete (*Borrelia burgdorferi*) in vector ticks (*Ixodes dammini*). *Am J Trop Med Hyg* 1990; **42**: 352–357.
179. Steere AC, Sikand VK, Meurice F *et al.* Vaccination against Lyme disease with recombinant *Borrelia burgdorferi* outer-surface lipoprotein A with adjuvant. Lyme Disease Vaccine Study Group. *N Engl J Med* 1998; **339**: 209–215.
180. Sigal LH, Zahradnik JM, Lavin P *et al.* A vaccine consisting of recombinant *Borrelia burgdorferi* outer-surface protein A to prevent Lyme disease. Recombinant Outer-Surface Protein A Lyme Disease Vaccine Study Consortium. *N Engl J Med* 1998; **339**: 216–222.